Bisulfite Conversion protocol –using EZ DNA methylation kit from Zymo Research

Reagent preparation

1. Prepare CT conversion reagent – Add 750 µl water and 210 µl of M-Dilution buffer to CT conversion reagent, or thaw frozen stock stored at -20˚C
2. Vortex at room temperature for 10 minutes
3. Prepare M-Wash Buffer (if not done) – Add 24 ml of 100% ethanol to 6ml of M-Wash buffer concentrate

Conversion Protocol

4. Add 5µl of M-Dilution Buffer to the dna sample (best if sample is 200-500ng) and adjust total volume to 50 µl with water. Mix sample gently.
5. Incubate sample at 37˚C for 15 minutes
6. Add 100µl of prepared CT conversion reagent to each sample and mix
7. Incubate sample in thermocycler at 50˚C for 12-16 hrs (must be dark, CT conversion reagent is sensitive to light)
8. Incubate the sample at 4˚C for 10 minutes
9. Add 400 µl of M-Binding Buffer to a Zymo-spin IC column and place column into provided collection tube
10. Load sample into zymo-spin column containing M-binding buffer. Close cap and mix by inverting several times
11. Centrifuge at full speed (≥10,000 x g) for 30 seconds. Discard flow-through
12. Add 100 µl of M-Wash buffer to column. Centrifuge at full speed for 30 sec
13. Add 200 µl of M-Desulphonation Buffer to the column and let stand at room temperature for 15-20 min. After incubation centrifuge at full speed for 30 seconds.
14. Add 200 µl of M-Wash buffer to the column. Centrifuge at full speed for 30 seconds. Add another 200µl of M-Wash Buffer and centrifuge for an additional 30 seconds.
15. Place column into 1.5 ml centrifuge tube. Add 10µl of M-Elution Buffer to column. Centrifuge for 30 seconds at full speed to elute DNA.
16. Store DNA at -20˚C if not used right away.